

BIODEGRADATION OF CHLOROBENZENES UNDER AEROBIC AND ANAEROBIC CONDITIONS IN MODEL SYSTEMS

By

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ABSTRACT

Chlorobenzenes are one major class of pollutants in the water environment. Because chlorobenzenes have been produced extensively in the past century, this brings some spillage accidents throughout history, and the Standard Chlorine of Delaware Superfund site is one of them.

The final goal of this research is to develop and investigate a bio-barrier to remove chlorobenzenes from the slow moving ground water contaminated by chlorobenzenes. In this thesis, the experimental setup was tested and optimized. Methods of detecting concerned chemicals in the experiment was explored and developed; methods of maintaining the anaerobic condition in the influent was explored and developed; modeling of the oxygen concentration distribution and the chlorobenzene concentration distribution in the column was done using COMSOL Multiphysics software.

Advisor: Dr. Edward Bouwer

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CHAPTER 1 INTRODUCTION

Chlorobenzenes are a group of dense non-aqueous phase liquid chemicals, usually synthesized artificially and widely used in the industry of manufacturing herbicides, dyestuffs, rubber, and as common intermediates in manufacturing other chemicals. These solvents are also known as high-boiling solvents in many applications of industry as well as in the laboratory.

Because of the numerous usages in industry and laboratory, chlorobenzenes were produced extensively in the past century (Yadav, Wallace, & Reddy, 1995). This resulted in some spillage accidents throughout the past several decades. Annually, hundreds of tons of chlorobenzenes have been spilled onto the soils or into the waters. Since chlorobenzenes are dense non-aqueous phase liquids chemicals, they tend to sink below the water table when there is a spillage in significant quantities and only stop when they reach impermeable bedrock (Saines, 1996). In this case, their penetration into an aquifer makes them difficult to locate and remediate, resulting in contaminating environments and ecosystem in the world (Jackson, 2004). Furthermore, the contamination caused by chlorobenzenes can last decades, especially to groundwater, as the low solubility of chlorobenzenes and the slow movement of groundwater.

In addition, chlorobenzenes are one of the groups known as carcinogens, many of which can be found on the U.S. Center for Disease Control's Hazardous Substance Priority List. For example, monochlorobenzene exhibits moderate toxicity to humans as indicated by its LD₅₀ of 2.9 g/kg (Rossberg et al., 2006). Meanwhile, groundwater is one of the most significant water sources for human drinking systems and ecosystems. As a result, the groundwater polluted by chlorobenzenes brings a huge risk to human health as well as the environment.

From 1966-2002, chlorobenzenes were manufactured at the former Standard Chlorine of Delaware plant, which was designated as a Superfund site by the U.S. Environmental Protection Agency (EPA). During their operation, hundreds of thousands of gallons of pure chlorobenzenes were accidentally spilled from storage tanks into the adjacent sediment and groundwater, resulting in high levels of chlorobenzenes being detected in the groundwater flowing through adjacent wetlands and into the Delaware River watershed. Because the natural remediation of chlorobenzenes is so slow, the presence of the chlorobenzenes has brought a long-term threat of chronic exposure.

In situ bioremediation of harmful chemicals is an effective and inexpensive way comparing with pump and treat methods, especially when the contaminants are completely mineralized (Vogt, Simon, Alfreider, & Babel, 2004). Several kinds of microorganisms were reported to have the ability to rapidly biodegrade chlorobenzenes (Dermietzel & Vieth, 2002) (Balcke, Turunen, Geyer, Wenderoth, & Schlosser, 2004). Aerobic or anaerobic conditions can be the major factor influencing the effectiveness of the biodegradation processes, as well as the types of electron acceptors (Warren & Haack, 2001). Several kinds of microorganisms which have the ability to degrade chlorobenzenes have been separated and identified from polluted water and soil in recent decades. For example, the bacterium *Rhodococcus phenolicus* degrades chlorobenzene as a sole carbon source (Rehfuss & Urban, 2005). The major biodegradation of chlorobenzenes is through the aerobic condition; the biodegradation of chlorobenzenes in the anaerobic condition is relatively quite slow (Frasconi, Zanolli, & Danko, 2015).

In this study, an *in situ* reactive bio-barrier, which was designed by Ph.D. student Steven Chow at Johns Hopkins University, was developed and investigated to remove chlorobenzenes from the slow moving ground water. At the early stage of this study, batch experiments were

conducted to explore the biodegradation of monochlorobenzene, dichlorobenzene and trichlorobenzene with an aerobic culture (15-B) under aerobic condition and an anaerobic culture (WBC-2) under anaerobic condition as well as the specific degradation mechanisms. Also, different electron acceptors were tried in the batch experiments. Then, a column system filled with sand as a growth matrix for microorganisms was set up to simulate the real contaminated site situation. As the contaminated groundwater passes through both anaerobic and aerobic environments, respectively, towards the surface, the column contains an oxygen gradient from anaerobic to aerobic created by external oxygen pumping. Key variables, such as methane production, concentration of chlorobenzenes and chloride ion, dynamic hydraulic properties, etc., are measured to evaluate the performance of the column. At last, COMSOL Multiphysics was used to model the physical properties of the column. The modeling of the distribution of oxygen concentration and chlorobenzenes concentration in the flow could help with the sampling. This software could be used in the future study to model the biodegradation of the chlorobenzenes and maybe have the potential to transform current modeling to *in situ* treatment of other halogenated organic solvents.

CHAPTER 2 LITERATURE REVIEW

Chlorobenzenes are one of the major pollutants in the water environment. The United States Environmental Protection Agency (USEPA) has listed monochlorobenzene, 1,2-dichlorobenzene, 1,3-dichlorobenzene, 1,4-dichlorobenzene, 1,2,4-trichlorobenzene and hexachlorobenzene as priority pollutants. As chlorobenzenes are usually synthesized artificially and widely used in the industry of manufacturing, normal microorganisms in the environment do not have the enzyme to degrade them; as a result, they use to be considered as difficult being biodegraded. The normal degradation method used to be chemical and physical method. However, the limitations of these methods are complex operation conditions and high cost. In the recent decades, some microorganisms found in the chlorobenzene polluted site had the ability to degrade chlorobenzenes. These organisms have been separated and identified from water and soil (Fennell, Nijenhuis, Wilson, Zinder, & Häggblom, 2004; van der Meer, Werlen, Nishino, & Spain, 1998).

In the aerobic chlorobenzene biodegradation, one of the most significant steps in the degradation of chlorobenzene is dechlorination. The C-Cl bond in the chlorobenzene is not considered activated. The activation of C-Cl bond needs high activation energy and a strong attacking nucleophile, which are hard to find in the organisms. Certain conditions can make the C-Cl bond not stable.

There are usually two pathways of biological dechlorination: 1. Ring cleavage first and then dechlorination; 2. Dechlorination first and then ring cleavage. One of the examples of the first kind of pathway is that *Pseudomonas* WR1306 can use monochlorobenzene as the only carbon source (Reineke & Knackmuss, 1984). The indigenous microbial community of the groundwater

degraded monochlorobenzene mainly via the modified ortho-pathway (Balcke et al., 2004). Its degradation pathway is shown below in the figure.

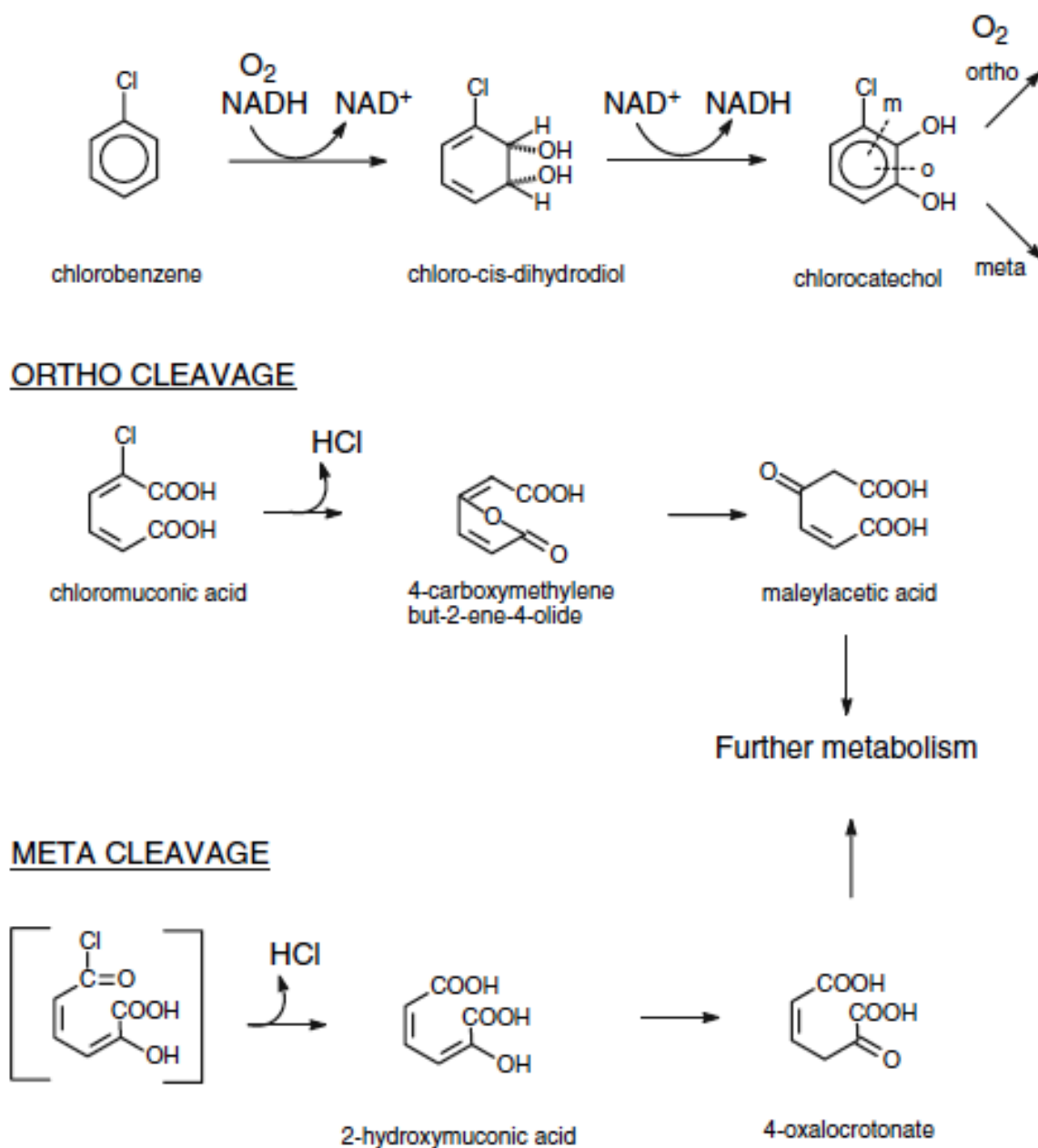


Figure 1 Pathways of aerobic biodegradation of CBs (Reineke and Knackmuss 1984, de Bont, Vorage et al. 1986, Sander, Wittich et al. 1991, Kaschabek and Reineke 1992, Mars, Kasberg et al. 1997).

One of the examples of the second kind of pathway is that of *Burkholderia* sp. PS12 that can use a dioxygenase to transform 1,2,4,5- tetrachlorobenzene (Beil, Happe, Timmis, & Pieper, 1997; Lehning, Fock, Wittich, Timmis, & Pieper, 1997). Its degradation pathway was shown below in the figure.

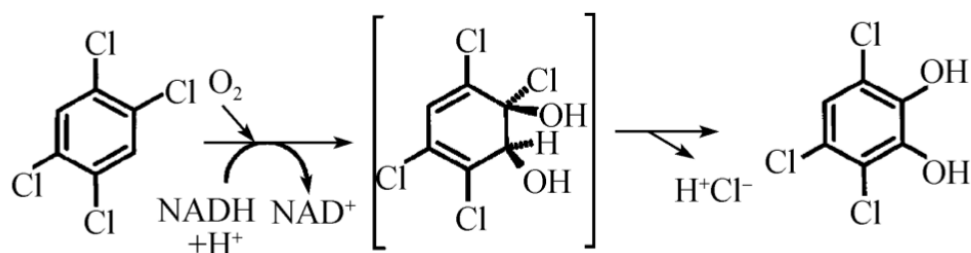


Figure 2 Dioxygenolytic dechlorination of 1,2,4,5- tetrachlorobenzene by *Burkholderia* sp. PS12

The dechlorination ability of the aerobic organisms decreases as the number of the Cl substitute on the benzene ring. Under aerobic conditions, chlorobenzenes with four or less chlorine groups are susceptible to oxidation by aerobic bacteria (*Burkholderia*, *Pseudomonas*, etc.) It was reported that mixed cultures usually have better effect on dechlorinating chlorobenzenes, because of cooperative metabolism (Jechorek, Wendlandt, & Beck, 2003).

In the anaerobic chlorobenzene biodegradation, the mechanism of anaerobic chlorobenzene biodegradation is that the chlorinated aromatic compounds receive two electrons and then release one chloride ion. The source of the electrons may come from outside organic compound, like formic acid, acetic acid, pyruvic acid, lactic acid, etc (Dolfing & Tiedje, 1991; Perkins, Komisar, Puhakka, & Ferguson, 1994), and the examples of this kind of microorganisms are *Dehalobacter*, *Dehalococcoides*, and *Dehalogenimonas* (Field and Sierra-Alvarez 2008); another source is endogenous respiration of the microorganisms. Some high chloride substituted benzenes can be easier dechlorinated under anaerobic conditions. Higher chlorobenzenes are

readily reductively dechlorinated to lower chlorinated benzenes in anaerobic environments. The pathways of anaerobic reductive dechlorination are shown below in the figure.

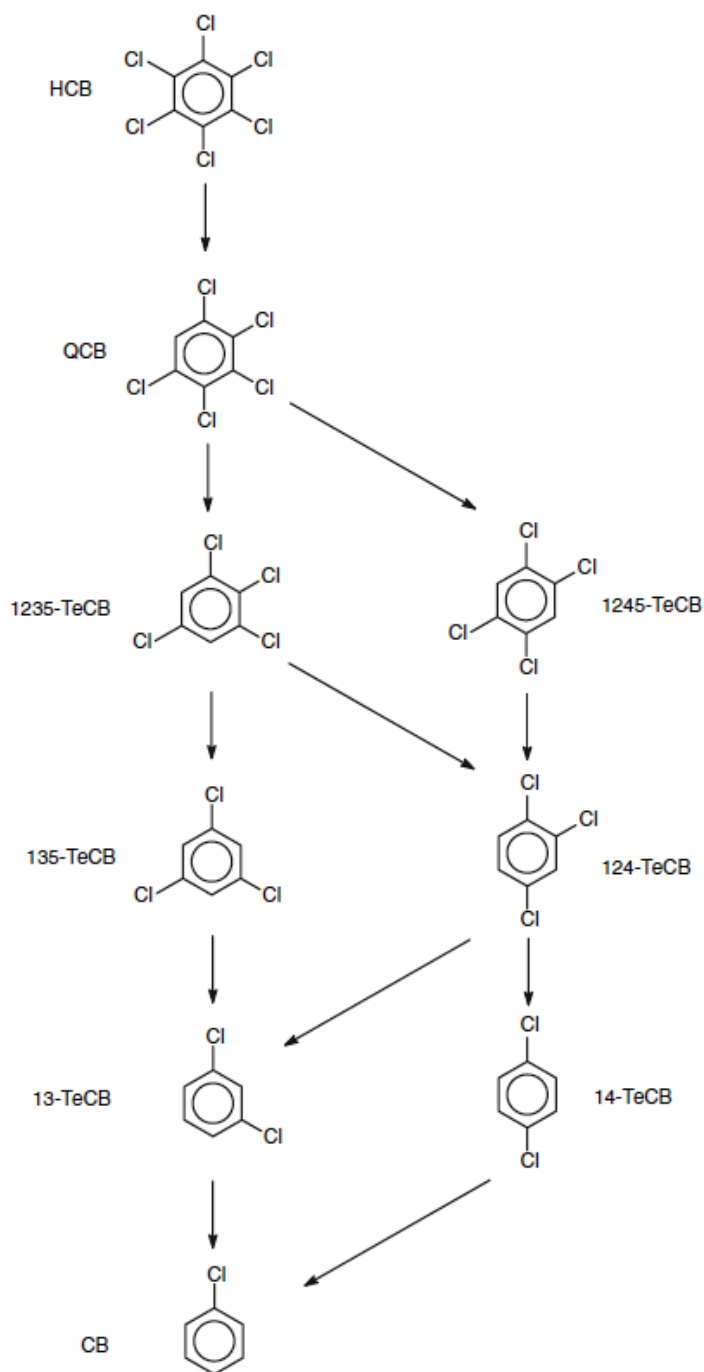


Figure 3 Pathways of anaerobic reductive dechlorination (Fathepure, Tiedje et al. 1988, Haigler, Pettigrew et al. 1992, Beurskens, Dekker et al. 1994, Middeldorp, De Wolf et al. 1997, Adrian, Manz et al. 1998, Chang, Su et al. 1998, Chen, Chang et al. 2002)

The idea of using fixed biofilm to treat organic pollutants has been raised up decades ago. McCarty et al. found that the biological removals of chlorinated benzenes and aromatic hydrocarbons on expanded GAC by adsorption and bacterial activity was much quicker than bacterial activity alone (Bouwer & McCarty, 1982). The reactive bio-barrier that combines physical sorption processes with biological degradation was reported stable and reliable (McGuire & Suffet, 1981). Using activated carbon as a sorptive growth matrix can increase carbon loadings and the removal of organic compounds as the refractory substances can storage on the GAC (McGuire & Suffet, 1981). Within biofilms attaching to aquifer minerals, oxygen gradients may become established (Balcke et al., 2004).

Our research is to try to set up a novel, *in situ* bio-barrier that can remove chlorobenzenes from slow-moving groundwater. When the polluted groundwater is discharged towards the surface at the contaminated site, it passes through anaerobic and aerobic environments respectively. In the anaerobic zone, high Cl^- substituted chlorobenzenes will be transformed to lower Cl^- substituted chlorobenzenes. As water goes up to the surface, the concentration of the dissolved oxygen increases and this will help the aerobic microorganism transform lower chlorobenzenes to H_2O , H^+ , and Cl^- .

CHAPTER 3 MATERIALS AND METHODS

In this chapter, all the major materials used in the experiments are stated as well as sampling and detection methods. At the end of this chapter, the background and theory of the physical modeling of the column are presented.

3.1 Materials

3.1.1 Media

The media was used for feeding the inoculum microorganisms and as the simulated chlorobenzene polluted water in the simulated barrier continuous-flow column system. The media used was simulated to the real groundwater. Three major categories of components in the media were groundwater macronutrients, trace elements, and vitamin solution. The components of each stock solutions are listed below in the tables.

Table 1 Compositions of groundwater macronutrients stock solution

Groundwater Macronutrients							
Component	KH ₂ PO ₄	K ₂ HPO ₄	Na ₂ HPO ₄	CaCl ₂	FeCl ₃ •6H ₂ O	NH ₄ Cl	MgSO ₄ •7H ₂ O
Concentration (mg/L)	8.5	22	33	28	1.25	250	61.625

Table 2 Compositions of trace elements stock solution

Trace Elements							
Component	MnSO ₄ •4H ₂ O	(NH ₄) ₆ Mo ₇ O ₂₄ •4H ₂ O	Na ₂ B ₄ O ₇ •10H ₂ O	CoCl ₂ •6H ₂ O	CuCl ₂ •2H ₂ O	ZnCl ₂	NaVO ₃
Concentration (mg/L)	1	0.25	0.25	0.25	0.25	0.25	0.1

Table 3 Compositions of vitamin solution stock solution

Vitamin Solution								
Component	pyridoxi ne-HCl	Thiami ne-HCl	Ribofl avin	Nicotinic acid	Biotin	Folic acid	Cobala min	p- aminoben zoic acid
Concentratio n (mg/L)	0.1	0.05	0.05	0.05	0.05	0.02	0.05	0.05

For the media preparation, 3.5 L milliQ water (milliQ water system, Manufacturer: Milipore) was added into a 4 L glass bottle after thoroughly rinsing, autoclaved for 45 min at 121 °C under liquid cycle. After that, N₂ was purged into the liquid for at least 2 hours to remove O₂. Macronutrients, trace metals, and vitamins stock solutions were added under 1000 dilution ratio to the media, and then 8 mg/L of neat 1,2,4-TCB was added by micro-syringe in fume hood as well. The feed for aerobic condition was directly stirred, and the feed for anaerobic condition was kept in the anaerobic glove hood to be stirred. Both kinds of feed were stirred for 2 days before being used. Before the feed replacement for microorganisms, 15 mmol/L lactate solution was added into the anaerobic feed by volumetric cylinder as electron donor (Vogt et al., 2004).

3.1.2 Microorganism

The microorganisms 15-B used in the experiment came from filtered groundwater at the wetland adjacent to the contaminated Standard Chlorine of Delaware Superfund site. These microorganisms showed the ability of degrading chlorobenzenes in the groundwater in the natural ecosystem.

The enrichment of aerobic 15-B culture was based on the continuous feed and stirred reactor in semi-continuous mode. The hydraulic retention time for this continuous stirred reactor

was 28 days. Every 3.5 days, 250 mL liquid from the culture was replaced by 250 mL fresh feed. The cultivated culture would be diluted by exactly the same volume of feed when the absorbance reached 0.1 (Seigneur, Vuillemin, Adler, & Peringer, 2001).

The growth of the microorganisms was measured by UV-Visible spectrophotometer (Spectrophotometer UVVis, manufacturer: Shimadzu) at 600 nm. The feed used for cultivating the microorganism contained the same macronutrients, trace metals, and vitamins composition, but the chlorobenzene source came from a continuous stream of sterile-filtered air going through neat liquid MCB and 1,2-DCB to evaporate them. The composition ratio of the neat liquid MCB, 1,2-DCB was 6:1. The flow rate was not measured, but should be slow enough to evaporate the chlorobenzenes (Liu et al., 2001).

The anaerobic degrading consortium WBC-2 used in the experiment was isolated by one of the collaborators at the United States Geological Survey (USGS) from a previous field study investigating degradation of chlorinated ethenes. This culture was obtained from SiREM labs, a commercial laboratory that maintains a continuous culture supply. This culture was stored in the anaerobic chamber (manufacturer: Coy, palladium catalyst and hydrogen gas mix of 5%).

3.1.3 Anaerobic condition maintenance

The microorganism WBC-2 culture needed to be grown in an anaerobic environment, and some of the experimental conditions as well as anaerobic feed needed to be maintained in an anaerobic environment. In this case, a vinyl anaerobic chamber was used (manufacturer: Coy, palladium catalyst and hydrogen gas mix of 5%). The protocol of putting in and removing something was vacuuming and filling in the attached box with N₂ twice and vacuuming and filling in the attached box with H₂ once.

3.2 Analytical methods

3.2.1 Methane detection method

Methane detection was quite important, as the production of the methane helped us to understand the mechanism of the biodegradation. Methane indicated highly reduced conditions which would indicate chlorobenzene degradation would be thermodynamically favorable. The existing methane protocols design for dissolved methane can be applied to column effluent samples (LeBeau, Montgomery, Miller, & Burmeister, 2000). The liquid samples sealed in glass vials were placed in glass vials for 15 min to let the methane in the head space and in the liquid reach equilibrium. In this case, the methane concentration originally in the liquid phase could be calculated based on the Henry's law.

The gas in the head space was tested by a gas chromatograph with flame ionization detector (manufacturer: Agilent 7890A/7683B AS) in the splitless mode. The temperature of the gas chromatograph oven was kept at 40 °C, and the holding time in this detection for methane was 5 min. Helium was the carrier gas and the flow rate of the gas was 2 mL/min. The composition of gas in flame ionization detector was 30 mL/min of H₂, 400 mL/min of compressed air, and 12 mL/min of makeup N₂ gas. The column used was DBFFAP 122-3232 (30 m x 250 µm, 0.25 µm film thickness, maximum temperature 250 °C).

Standard gas with known methane concentration made from pure methane gas and N₂ was used in the calibration. The pressure of the standard gas was maintained 1 atm. Known volume pure methane gas was injected into the glass serum bottles filled with pure N₂ gas and crimped with Teflon septa after the same amount of N₂ gas was pulled out. Gastight glass syringes were used in pulling out the N₂ gas and spiking methane.

3.2.2 Sugar detection method

The total sugar was measured using the phenol-sulfuric acid method. The sample used here was 0.5 mL; 0.5 mL 4% phenol and 2.5 mL 96% sulfuric acid were used as reagents, which were added into the samples respectively. After mixing, the samples were kept stated for 15 min to cool down. Then, the liquid was measured by UV-Visible spectrophotometer (Spectrophotometer UVVis, manufacturer: Shimadzu) at 490 nm (Disney, Zheng, Swager, & Seeberger, 2004).

The 10 g/L glucose standard solution was used in the calibration. The detection range here was 0-0.3 g/L. The detailed concentration of the standards and absorbance were shown below in the table and the calibration curve was shown in the figure as well.

Table 4 Glucose concentration and absorbance in the calibration with a detection range of 0.1-0.3 g/L

Concentration (g/L)	0	0.10	0.16	0.20	0.24	0.30
Absorbance	0.1414	0.8682	1.3517	1.4970	1.8972	2.2628

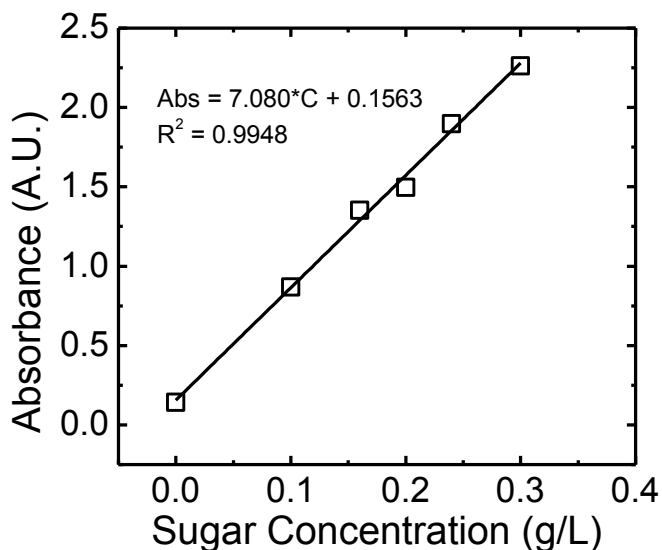


Figure 4 Total sugar detection calibration curve with a 0.1-0.3 mg/L detection range

Besides the standard method mentioned above, alteration of the volume of sample, phenol and sulfuric acid was also made to try to figure out a more accurate way to measure total sugar in the low concentration.

3.2.3 Dissolved oxygen detection method

Dissolved oxygen was measured using a Hach multi-measurement instrument and applying to the Luminescence method. The dissolved oxygen probe was calibrated in the head space of a bottle of water with saturated water vapor.

3.2.4 Anions detection method

The analysis of some anions of interest was very important to help understand the degradation of different kinds of chlorobenzenes and help us to understand the mechanism of this biodegradation. The important anions included lactate, nitrate, sulfate, and chloride. In this case, an ion chromatograph with a conductivity detector (manufacturer: Thermo Scientific (Dionex)) was used to separate and quantify these anions. In the test, the samples were diluted 10 times to ensure the results falling into the detection range and having enough liquid to be tested. Samples were analyzed isocratically using 30 mM KOH as the eluent.

3.2.6 Flow rate measurement

The flow rate was calculated as the fluid volume of the effluent dividing by the time of collecting the effluent. The volume of the effluent was determined by weighing the difference of the bottle before and after the effluent collection. As the effluent was a low concentration solution, the density of the effluent could be estimated as the same as liquid water.

3.3 Simulated barrier continuous-flow column system setting up

The goal of this long-term column study, based on the earlier batch experiments, is try to simulate the real remediation situation, so that the results of this study could be applied to the Standard Chlorine of Delaware site and transform the current study to *in situ* biodegradation treatment of other halogenated organic solvents.

3.3.1 Column experiment design

In this part of the study, different inoculums were tested under aerobic and anaerobic conditions respectively. Six different biofilm columns were designed by Ph.D. student Steven Chow. The detailed column design is shown in the table below.

Table 5 Experiment design for biofilm column study by Ph.D. student Steven Chow

No	Oxygen Condition	Anaerobic inoculum	Aerobic inoculum
1	Anaerobic	-	-
2	Anaerobic	WBC-2	-
3	Anaerobic	WBC-2	15-B
4	Anaerobic	WBC-2	15-B
5	Anaerobic	-	15-B
6	Aerobic	-	15-B

Column number 1 was treated with additional 200 mg/L sodium azide (NaN_3) to prevent the growth of microorganisms as the control group. Column number 1 through column number 5 were treated by anaerobic feed to maintain the anaerobic condition and column number 6 was treated by aerobic feed. Besides, external N_2 was connected at the top of column number 1 through column number 5 to ensure the anaerobic condition. The components of the feed have been listed in the media above.

Sand was used as a growth matrix for microorganisms. No sorptive matrices were added in these columns to exclude the effects of abiotic sorption on biodegradation. Microorganisms WBC-2 and 15-B were inoculated to the growth matrix and feed was provided continuously for them growing on the sand and forming biofilm. After the inoculation, the simulated chlorobenzene polluted water flowed into the column from the bottom, passed through the biofilm, which attached on the sand media, and then flowed out from the top of the columns. Inflows and outflows were tested each day during the experiment. Important parameters included pH, flow rate, dissolved oxygen, concentration of methane, concentration of different kinds of chlorobenzenes, concentration of concerned anions, including Cl^- and SO_4^{2-} . The detailed measurement and detection methods has been mentioned in the detection method.

3.3.2 Feed replacement for column experiment

The feed replacement period for the columns was 5 days. During the replacement of the feed, the N_2 bag connected to column number 1 through number 5 was also replaced to ensure the anaerobic environment and prevent displaced liquid.

3.3.3 Samplings and parameters tested in the column experiments

The column sampling including influent and effluent for DO and pH test was everyday with two duplicated samples from each column; the column sampling of influent for GC-MS and IC analysis was every other day with two duplicated samples and the column sampling of effluent for GC-MS and IC analysis was everyday with two duplicated samples.

3.3.3.1 Flow rate measurement and samplings for dissolved oxygen and pH test

The flow rate of the columns was measured by weighing the mass difference of small glass vials after effluents filled in the glass vials for 1 hr. The density of the sample was taken as same

as the water because of the fairly low concentrations of solutes in the solution. Based on this, the volume of the solution could be calculated. As a result, the flow rate of the columns could be calculated as volume of the solutions divided by time.

Also, the mouth of the small glass vials was big enough for pH and dissolved oxygen probes testing directly. For the dissolved oxygen measurement, Luminescence method was used and Hach multi-measurement instrument was the instrument used here. The dissolved oxygen probe was calibrated everyday before used. pH was measured using a semi-micro probe with calibration at pH 4, 7, and 10.

During the experiment of determining the liquid flow rate and the concentration of $\text{Na}_2\text{S}_2\text{O}_3$ to make the oxygen concentration in the liquid outlet reaching a low oxygen concentration, the dissolved oxygen was tested in the same way, but after the sample bottle was filled up 3 times to ensure the liquid in the sample bottle all coming from the same liquid flow rate and evacuating all the liquid in the tube. This experiment was trying to find a good way to maintain the anaerobic conditions in the anaerobic experiment.

3.3.3.2 Sampling for GC-MS and IC analysis

The everyday sampling was for the gas chromatography-mass spectroscopy and ion chromatography analysis. During the everyday column sampling, glass syringes after sufficient rinsing were put onto the Luer valves at the top of columns to collect effluent. The effluents collected in first 5 min were used to rinse the syringes and casted away to empty the liquid and gas in the syringes. After that, two 2 mL samples were recollected from each syringe respectively, as the testing was applied to two duplicates.

For the analysis of the concentrations of chlorobenzenes by chromatography-mass

spectroscopy, 1 mL cyclohexane was added ahead of sampling to the brown glass auto sample vials by pipette (Souissi et al., 2013). A 100 uL glass microsyringe was used to transfer 100 uL sample from the glass syringes into the GC-MS autosample vials. After the vials were capped, the vials were vortexed for 5 min for extraction, and then the samples were stored in 4°C for future GC-MS analysis.

For the ion chromatography analysis, 0.5 mL samples were diluted with 10 times dilution ratio to 5 mL plastic auto sampler vials.

3.3.3.3 Sampling for methane measurement

About 5 mL liquid samples from the effluents were injected into 26 mL glass serum bottles. The exact volume of the samples could be calculated by weighting the difference before and after the sampling. After sampling, there would be a 15-minute standing period to allow the methane in the liquid phase and the methane in the gas phase to reach equilibrium. Then, 100 μ L air was taken from the headspace by gastight microsyringes, and injected into the sample inlet port of the GC-FID manually.

The original methane concentration in the liquid phase could be calculated based on the Henry's Law and basic mass balance.

3.4 Anaerobic condition control for the influent

In the experiment, the influent dissolved oxygen of the anaerobic column needed to be approximately zero to prevent growth of aerobic organisms at the column entrance. In this case, several experiments were done to try to figure out a method.

NaS_2O_3 was used as a reactive oxygen scavenger O_2 here. Two different kinds of tube arrangements were used here: curled and uncurled. The curled tubes were wrapped around the tube to increase the contacts with solution. Three different concentrations of NaS_2O_3 were tested, including 0.1 M, 0.5 M and 1.0 M, and four different flow rates were tested here, including 0.823 mL/min, 1.62 mL/min, 2.73 mL/min and 3.13 mL/min. Also, using N_2 to purge the influent reservoir was also another variable.

In the experiment, the water in the influent reservoir was pumped through silicone tubing. The tubing was soaked in the NaS_2O_3 solution, where oxygen could diffuse into the oxygen-scavenging solution first before entering the pump, and then finally flowing to the sample collection bottle.

3.5 COMOSL Multiphysics modeling

COMSOL Multiphysics is a finite element analysis, solver and simulation software / FEA software package for various physics and engineering applications, especially coupled phenomena, or multiphysics.

COMOSL Multiphysics was used to model the physical properties of the column. The modeling of the distribution of oxygen concentration and chlorobenzenes concentration in the flow could help with the sampling. Because the distribution of the concentrations of the concerned

species was not homogeneous in the columns, the sampling should be somewhere that could represent the real result. Also, modeling could help choose suitable calibration tube and injection needles without too many experiments. Besides, the velocity magnitude, pressure drop, concentrations of concerned species could all be plotted.

Hopefully, this software could be used in the future study to model the biodegradation of the chlorobenzenes and maybe have the potential to transform current modeling to *in situ* treatment of other halogenated organic solvents.

3.5.1 Theories and Assumptions

The model used here is the porous reactor with injection needle, which was inspired by numerical experiments performed by Professor Finlayson's graduate students in chemical engineering at the University of Washington in Seattle.

This model could be applied to the column system because the main axis of the inlet oxygen tube was perpendicular to the main axis of the feed inlet. The sand in the main reactor, which the feed flowed through, could be considered as the porous media interface. Both free-flow domains and porous-media domains were supported as the interface.

In this modeling, the Brinkman equations were applied in the porous media. The Brinkman equations describe flow in porous media that is fast enough that the drive for flow includes kinetic potential related to fluid velocity, pressure, and gravitational potential. The Brinkman equations appear as a mix of Darcy's law and the Navier-Stokes equations. They extend Darcy's law to account for dissipation of kinetic energy by viscous shear as in the Navier-Stokes equation (Whitaker, 1986). Since the concentration of the concerned species in the experiments was quite

low compared to the concentration of the inlet oxygen, Fickian approach could be used in the mass transport in this situation. This approach describes diffusion but not mechanical dispersion, which fitted the column experiment situation.

When it comes to the boundary modeling of the columns, a constant velocity profile was applied at the inlet boundaries, while a pressure condition was applied to the outlet. Assume the gradient of the inlet concentration perpendicular to the outlet boundary could be negligible in the experiments. The concentration of the species at the inlets in the column system were fix and the convection dominated the mass transport at the outlet.

Only diffusion was applied in this modeling effort. In a future study, Arrhenius law could also be combined here, so that biodegradation reaction could also be modeled using the software.

3.5.2 Model construction for oxygen diffusion in the water in the column

According to the experiment situation, transport of diluted species (rfd) should be chosen under the flow in porous media in the chemical species transport mode. Oxygen gas and liquid water were the two concerned species in this modeling. The stationary mode was chosen as the column would be in the steady state.

3.5.2.1 Geometry definition

First, a cylinder with a radius of 12.5 mm and a height of 150 mm was defined as the column. Then, the same cylinder was defined again, so that later it could be defined as the place where the oxygen diffusion could happen. This big cylinder was defined as the column. After that, a cylinder hole, whose main axis was perpendicular to the main axis of the cylinder, was dipped out. The radius of this cylinder was 1.3 mm and the height was 12.5 mm, which should be the

same as the radius of the main cylinder. Then, a cylinder with a radius of 1.25 mm and a height of 15 mm was inserted into the cylinder hole and the main axis of this cylinder was perpendicular to the main axis of the column cylinder. This small cylinder was defined as the injection needle. In this case, the geometry of the column was set up. In order to view the diffusion of the oxygen more directly and clearly, the system was cut off from the plane which was constructed by the two main axes of this cylinder, which was perpendicular to each other. The geometry of the combination is shown below in the figure.

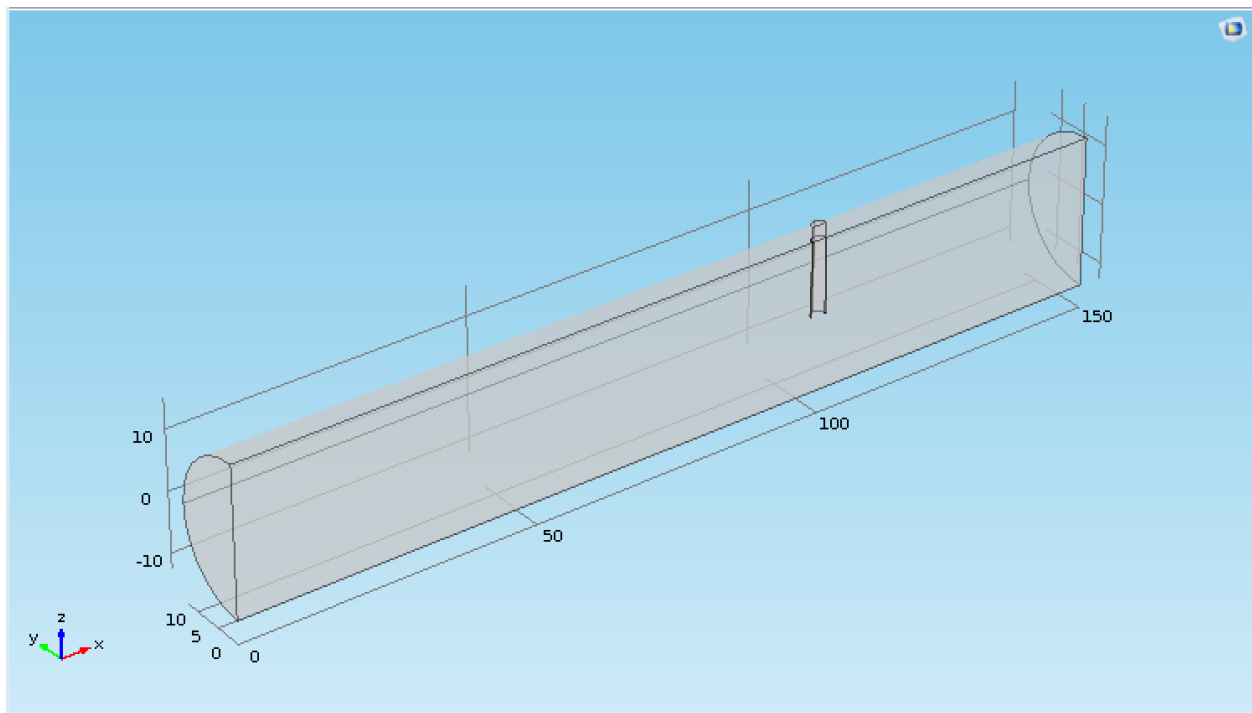


Figure 5 The geometry combination of the column

3.5.2.2 Definition of domains and boundaries

Each part of the combination was defined as a domain or boundary. As the cut off was only for display, the plane, which was constructed by the two main axes of these cylinders, was defined as geometric entry level instead of the boundary as it was not the real boundary. After that, the main cylinder was defined as the porous bed, where the oxygen diffusion would happen.

3.5.2.3 Definition of variables

First, the material of the carrier liquid was chosen as water. In the transport property setting, the diffusion coefficient D should be set as $2 \times 10^{-9} \text{ m}^2/\text{s}$, which was the oxygen diffusion coefficient in water. For the porous matrix properties, the porosity of the domain material was 0.3. The diffusion coefficient should be set as isotropic. The permeability was $3.68 \times 10^{-10} \text{ m}^2$, which was calculated by Darcy's law, which is an equation that describes the flow of a fluid through a porous medium, and Hagen–Poiseuille equation, which is a physical law that gives the pressure drop in an incompressible and Newtonian fluid in laminar flow flowing through a long cylindrical pipe of constant cross section (Whitaker, 1986). The permeability coefficient should also be set as isotropic.

The location of water inlet was defined on one of the ends of the main cylinder. The inflow velocity was 0.0000027 m/s , which was calculated by flow rate measured and the area of the inlet plane. The second inlet was the oxygen gas. The location of the oxygen inlet was defined at the top of the injection needle. The inflow oxygen velocity was $1.69 \times 10^{-5} \text{ m/s}$, which was calculated by the oxygen flow rate and the diameter of the injection needle. The concentration of the oxygen was 0.625 mol/m^3 , which was calculated by the oxygen flow rate and ideal gas law. The location of the outlet was defined at the other end of the column. The boundary condition should be set as pressure instead of laminar outflow. At last, the plane, which was constructed by the two main axis of these cylinder perpendicular to each other, should be set up as the symmetry plane.

3.5.3 Model construction for chlorobenzene diffusion in the water in the column with oxygen injection

According to the experiment condition, transport of diluted species (rfd) should be chosen under the flow in porous media in the chemical species transport mode as the concentration of the chlorobenzene and oxygen were relatively low. Oxygen gas, chlorobenzene and liquid water were the two concerned species in this modeling. Here, the 1,4-dichlorobenzene was taken as a representative. The stationary mode was chosen as the column would be in the steady state. The modeling process was similar to the oxygen diffusion in the water stated above. The geometry definition was the same as the modeling of the oxygen diffusion in the water, and it would not be stated again here. The definition of domains and boundaries of this chlorobenzene diffusion in the water in the column with oxygen injection model was nearly the same, except that the chlorobenzene was in the influent and it would diffuse in the porous media as well.

First, the material of the carrier liquid was chosen as water. In the transport property setting, the diffusion coefficient D should be set as $2 \times 10^{-9} \text{ m}^2/\text{s}$, which was the oxygen diffusion coefficient in water. Because the concentration of the chlorobenzene was relatively low, the oxygen diffusion coefficient here could be taken as the same in the water. For the porous matrix properties, the porosity of the domain material was 0.3, which was the same. The diffusion coefficient should be set as isotropic. The permeability was $3.68 \times 10^{-10} \text{ m}^2$, which was calculated by Darcy's law, which is an equation that describes the flow of a fluid through a porous medium (Whitaker, 1986), and Hagen–Poiseuille equation, which is a physical law that gives the pressure drop in an incompressible and Newtonian fluid in laminar flow flowing through a long cylindrical pipe of constant cross section (Sutera & Skalak, 1993). The permeability coefficient should also be set as isotropic.

The location of the chlorobenzene solution inlet was defined on one of the ends of the main cylinder. The inflow velocity was 0.0000027 m/s, which was calculated by flow rate measured and the area of the inlet plane. The second inlet was the oxygen gas. The location of the oxygen inlet was defined at the top of the injection needle. The inflow oxygen velocity was 1.69×10^{-5} m/s, which was calculated by the oxygen flow rate and the diameter of the injection needle. The concentration of the oxygen was 0.625 mol/m³, which was calculated by the oxygen flow rate and ideal gas law. The location of the outlet was defined at the other end of the column. The boundary condition should be set as pressure instead of laminar outflow. At last, the plane, which was constructed by the two main axis of these cylinder perpendicular to each other, should be set up as the symmetry plane.

3.5.4 Modeling chlorobenzene biodegradation

Chlorobenzene biodegradation reaction could also be modeling based on the Arrhenius law, which gives the dependence of the rate constant of a chemical reaction on the absolute temperature, a pre-exponential factor and other constants of the reaction. When the frequency factor A_f and the activation energy E_a were gained by the experiment, the biodegradation could be modeled in the future.

CHAPTER 4 RESULTS AND DISCUSSION

4.1 Simulated barrier in column system

The concentrations of the standard could be calculated using the ideal gas law. The detailed volume, concentration of the standards and peak area were shown below in the table and the calibration curve was shown in the figure as well. The detection range here was 0.1-1.0 mg/L.

Table 6 Methane concentration and GC response in the calibration with a detection range of 0.1-1.0 mg/L

	Methane Spiked (uL)	Bottle Volume (mL)	Methane Concentration (mg/L)	Peak Area
Blank A	0	159	0	6.3
Blank B	0	159	0	6
Blank C	0	159	0	5.9
Standard 1A	25	159	0.103	41
Standard 1B	25	159	0.103	40.4
Standard 1C	25	159	0.103	40.7
Standard 2A	75	159	0.309	100
Standard 2B	75	159	0.309	115
Standard 2C	75	159	0.309	116
Standard 3A	150	159	0.618	233
Standard 3B	150	159	0.618	214
Standard 3C	150	159	0.618	233

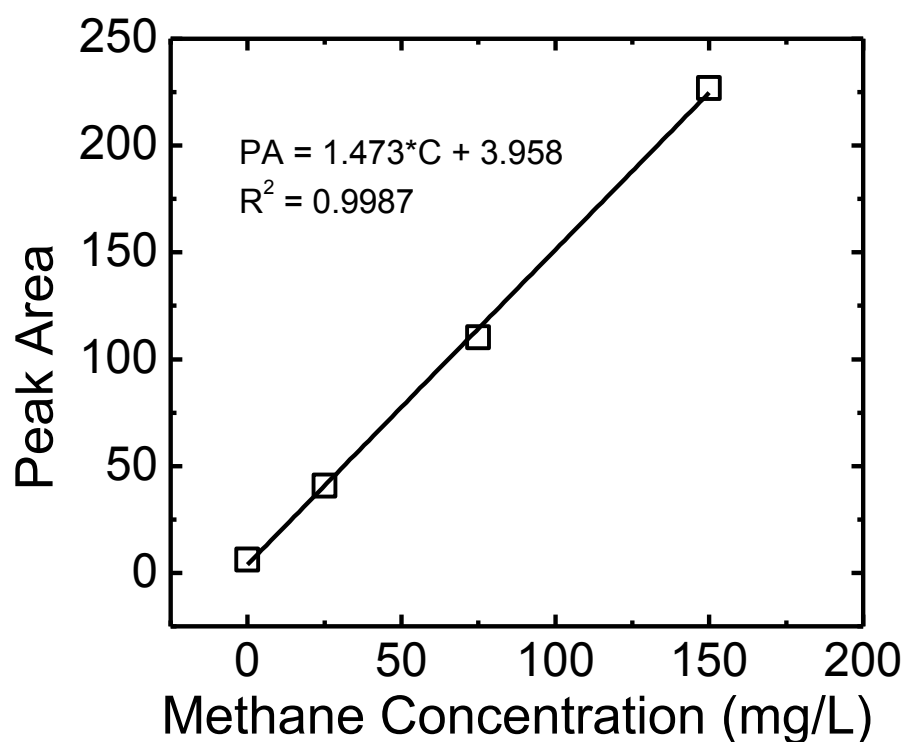


Figure 6 Methane detection calibration curve with a 0.1-1.0 mg/L detection range

Also, a calibration with the detection range of 2-20 mg/L was also done. The method of making the standard was the same as stated above. The detailed concentration of the standards and peak area were shown below in the table and the calibration curve was shown in the figure as well.

Table 7 Methane detection calibration curve with a 1.0-20 mg/L detection range

Concentration (mg/L)	Sample A Peak Area	Sample B Peak Area	Sample C Peak Area
Blank	0	0	0
2	6.58×10^2	6.57×10^2	6.76×10^2
5	1.37×10^3	1.39×10^3	1.39×10^3
20	6.66×10^3	7.03×10^3	6.99×10^3

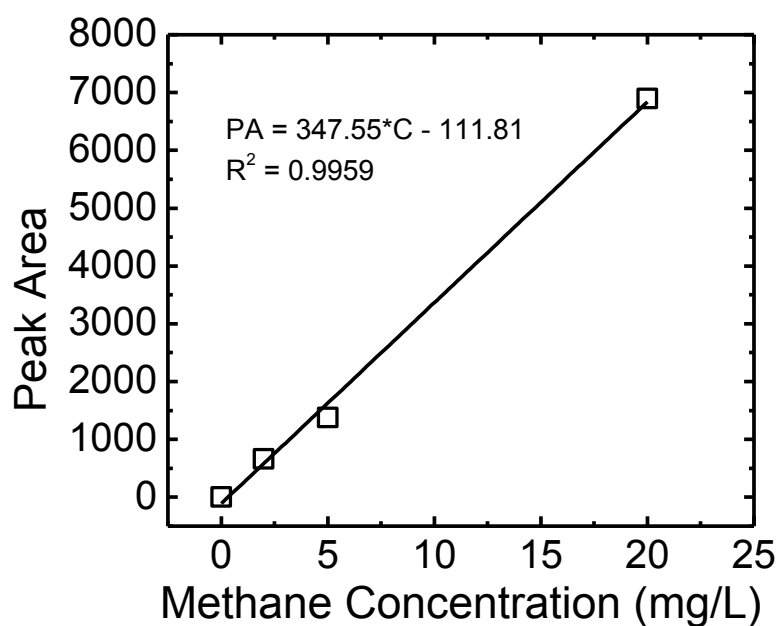


Figure 7 Methane detection calibration curve with a 1.0-20 mg/L detection range

Besides the above calibration with the detection range of 0.1-0.6 mg/L, another calibration with the detection range of 70-4100 ppm was also done. The method of making the standard was the same as stated above. The detailed concentration of the standards and peak area were shown below in the table and the calibration curve was shown in the figure as well.

Table 8 Methane detection calibration curve with a 70-4100 ppm detection range

Concentration (ppm)	Sample A Peak Area	Sample B Peak Area	Sample C Peak Area
75	77.6	79.1	69.9
189	1.08×10^2	1.09×10^2	1.08×10^2
491	2.63×10^2	2.65×10^2	2.61×10^2
1019	5.35×10^2	5.48×10^2	5.44×10^2
4088	2.37×10^3	2.31×10^3	2.30×10^3

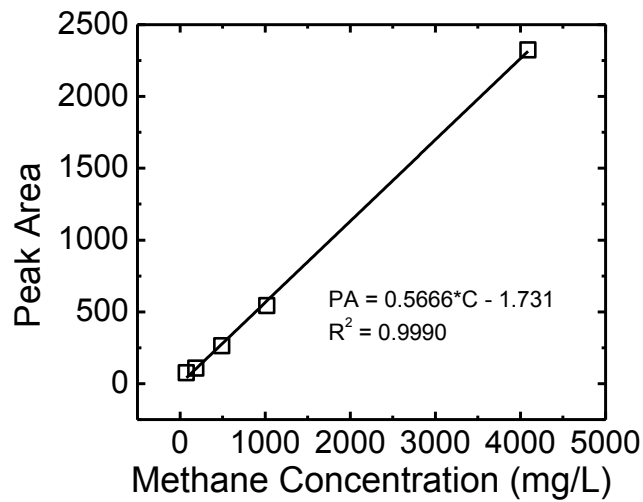


Figure 8 Methane detection calibration curve with a 75-4000 ppm detection range

4.2 Total sugar test

Total sugar test could help to quantify the biofilm. Using Phenol-sulfuric acid method to detect sugar is the common way. However, because of the approximate concentration of the total sugar in the experiment was lower than the typical detection range, further investigation was done to try to figure out the possibility of using total sugar test to quantify the biofilm.

The calibration curve was shown below in the figure.

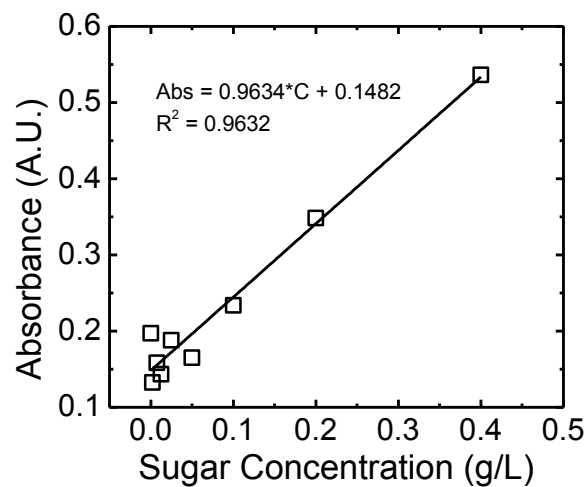


Figure 9 Total sugar detection calibration curve

From the figure, it is evident that the linear relationship between the sugar concentration and the absorbance was quite poor when the concentration of the sugar was lower than 0.1 g/L. Alteration of the volume of sample, phenol and sulfuric acid was also made to try to figure out a more accurate way to measure total sugar at the low concentrations. The original ratio was 1:1:5. Other ratios were also tried, including 1:2:3, 2:1:3, 1:1:4, 1:1:3. However, none of these ratios yielded an ideal calibration.

4.3 Anaerobic inflow test

4.3.1 Tube arrangement

NaS_2O_3 was used to absorb O_2 here. Two different kinds of tube arrangements were used here: curled and uncurled. The O_2 removal was plotted against different flow rate and the O_2 removal of the two kinds of tube arrangement was also shown in the figure.

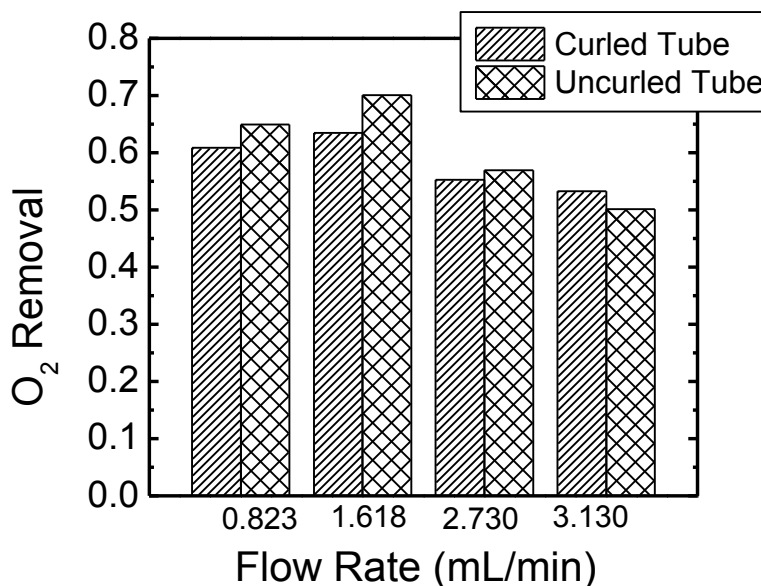


Figure 10 O_2 removal versus different flow rate and O_2 removal of the two kinds of tube arrangement

From the plot, it could be seen that at the flow rate of 1.618 mL/min, the O₂ removal reached the highest. The O₂ removal increased before the flow rate reached 1.618 mL/min and decreased after that. From the plot, it showed that around the low flow rate, the O₂ removal of the uncurled tube arrangement was even higher and around the high flow rate, the O₂ removal of the curled tube arrangement was higher. However, the difference was not significant. In this case, the uncurl pipe arrangement should be applied here in the further study as the materials could be saved in this case.

4.3.2 Concentration of NaS₂O₃ and flow rate

In this part, two factors, including different concentration of NaS₂O₃ and different flow rate, were investigated. The figure of the O₂ removal versus flow rate in different concentration of NaS₂O₃ was plotted below.

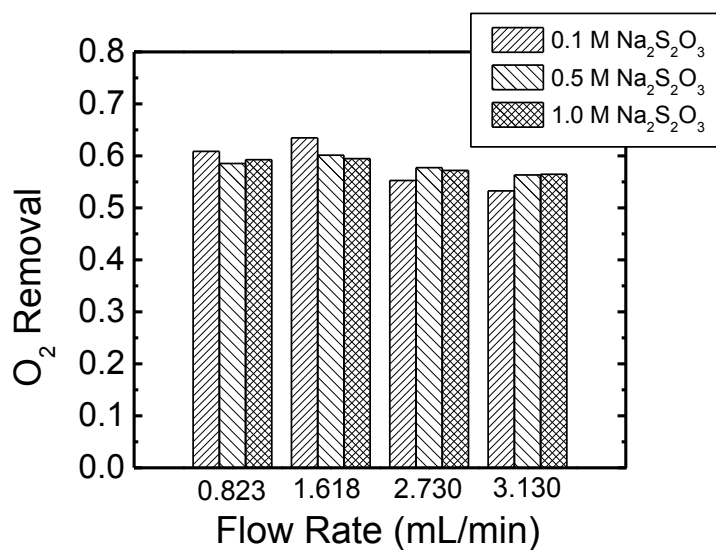


Figure 11 O₂ removal versus flow rate in 0.1 M, 0.5 M, 1.0 M NaS₂O₃

From the figure, it could be seen that at the flow rate of 1.618 mL/min, the O₂ removal reached the highest. The O₂ removal increased before the flow rate reached 1.618 mL/min and decreased after that, but the difference was not quite significant. About the concentration of

NaS₂O₃, the O₂ removal at 0.5 M NaS₂O₃ and at 0.5 M NaS₂O₃ was quite close to each other. The O₂ removal reached the highest when the concentration of NaS₂O₃ was 0.1 M NaS₂O₃ and the flow rate was 1.618 mL/min.

4.3.3 N₂ inlet before the NaS₂O₃ treatment

In this part, N₂ was inlet before the NaS₂O₃ treatment. The results were shown below.

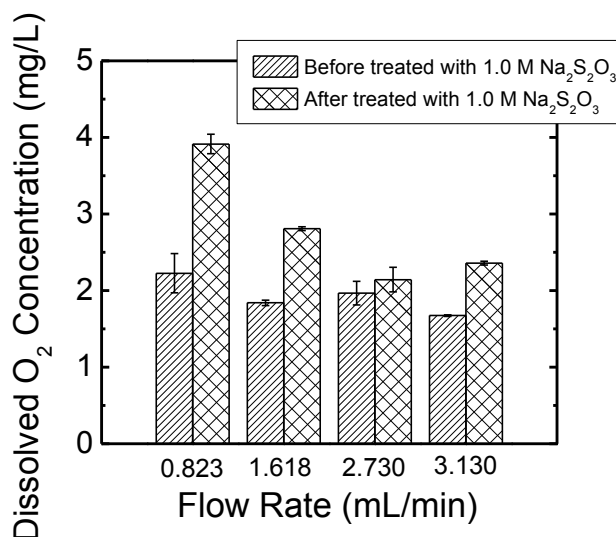


Figure 12 O₂ concentration versus flow rate when the pipe went to 1.0 M Na₂S₂O₃ solution first and then pumping system

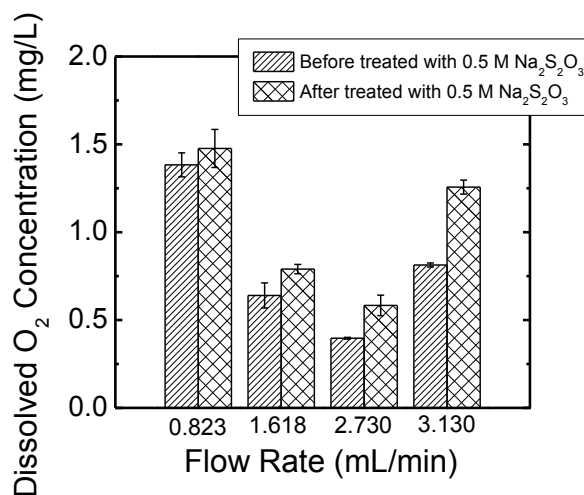


Figure 13 O₂ concentration versus flow rate when the pipe went to pumping system first and then 0.5 M Na₂S₂O₃ solution

From the figure, it could be seen that the concentration of dissolved oxygen even increased after the NaS_2O_3 treatment, which seemed not possible. Further investigation found that this may be brought by the pump and the connections.

Then, the location of the pump and the NaS_2O_3 solution was changed. The liquid went to the pump first and then went to the NaS_2O_3 solution. This time the connections of the pipes were put under the NaS_2O_3 solution and another contrast with connections out of the NaS_2O_3 solution was also done. The figure was plotted when the concentration of NaS_2O_3 was 0.1 M NaS_2O_3 and the flow rate was 1.618 mL/min.

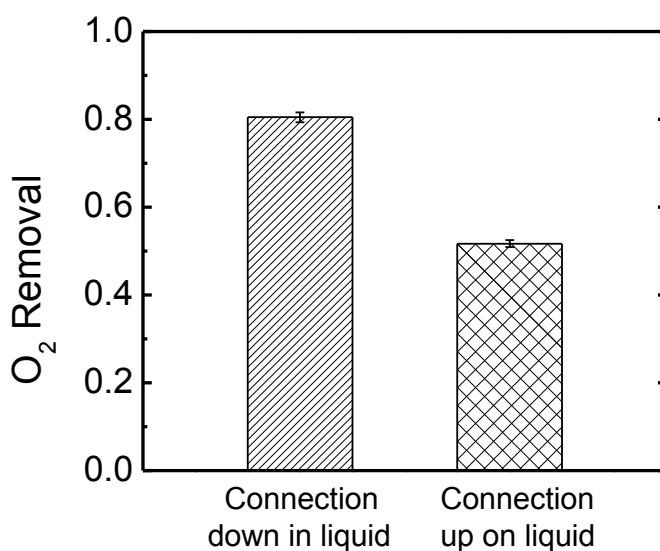


Figure 14 O_2 removal versus connection in and out of 0.1 M $\text{Na}_2\text{S}_2\text{O}_3$ solution at 1.618 mL/min flow rate

From the figure, it could be seen that this time the concentration of the dissolved oxygen decreased in both situations, which meant that the pump had a leak. Besides, the O_2 removal was higher when the connections of the pipes were put under the NaS_2O_3 solution, which meant that the connection was one of the leaking area too. In this case, when the concentration of NaS_2O_3 was

0.1 M $\text{Na}_2\text{S}_2\text{O}_3$ and the flow rate was 1.618 mL/min and the liquid went to the pump first and then went to the $\text{Na}_2\text{S}_2\text{O}_3$ solution and the connections of the pipes were kept under the $\text{Na}_2\text{S}_2\text{O}_3$ solution, the O_2 removal reached the highest.

4.4 COMSOL Multiphysics modeling

Using the COMSOL Multiphysics software, the distribution of the velocity, pressure, and the concentration of the dissolved oxygen in the column could be calculated and modeled using visual colors.

4.4.1 Oxygen diffusion in the water in the column

The distribution of the fluid velocity in the column was plotted in the figure below.

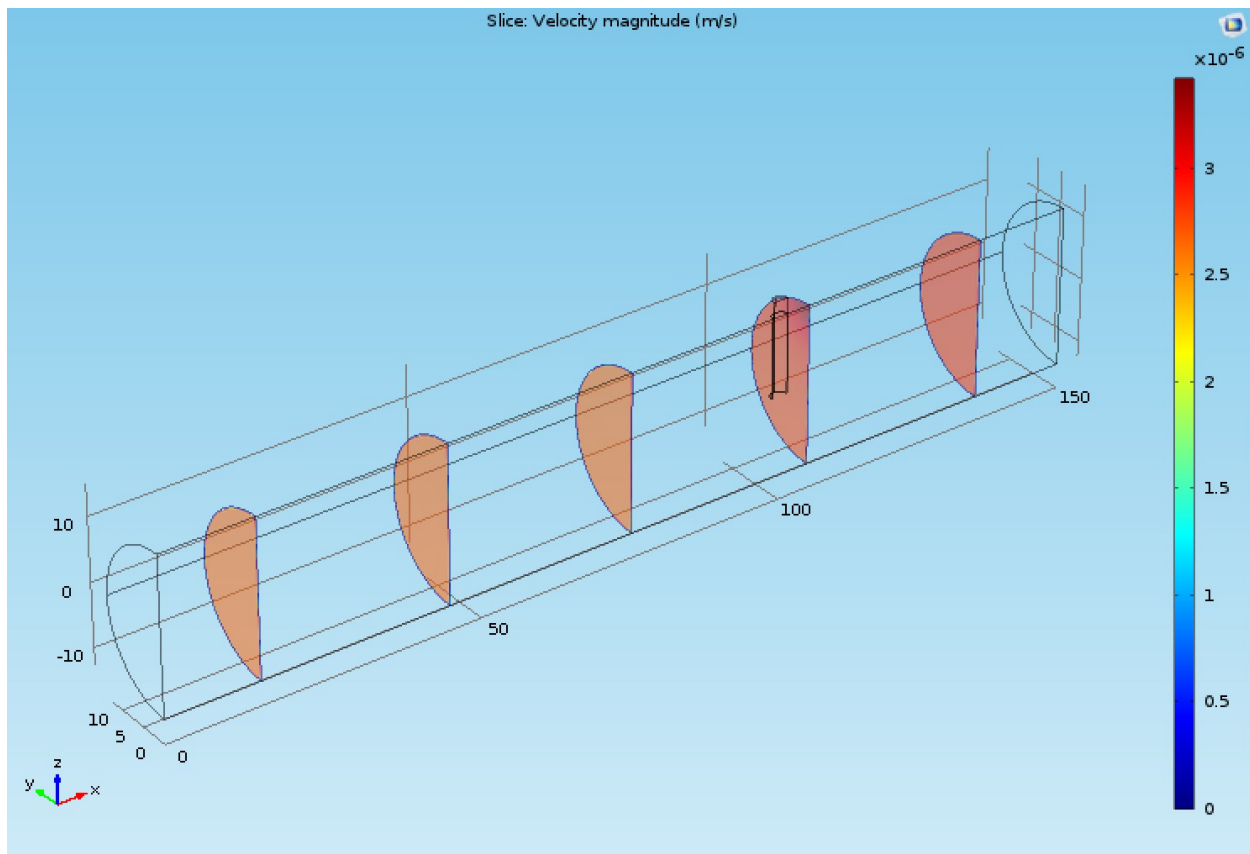


Figure 15 Distribution of the water velocity in the column with O_2 injection by 1.25 mm radius needle

From the figure, it could tell that the velocity of the fluid kept the same as the influent velocity until the fluid reach the injected needle area. The velocity of the fluid slightly increased as the additional oxygen was injected. In this case, the injected oxygen would not change the velocity of the fluid too much.

The pressure in the column was shown below in the figure.

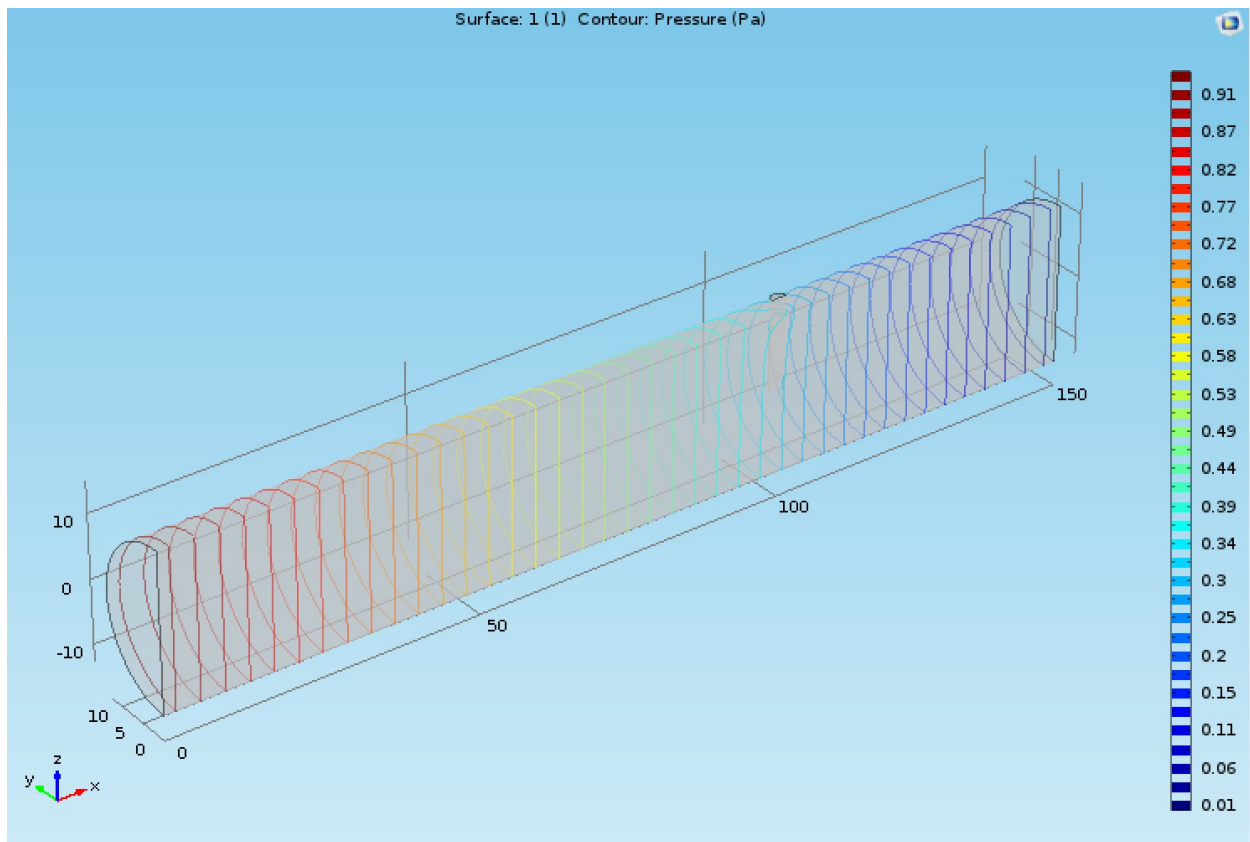


Figure 16 Pressure distribution in the column with O₂ injection by 1.25 mm radius needle

From the figure, it could tell that the pressure in the column decreased along with the liquid flow in the x direction.

The distribution of the oxygen concentration was shown in the below figure.

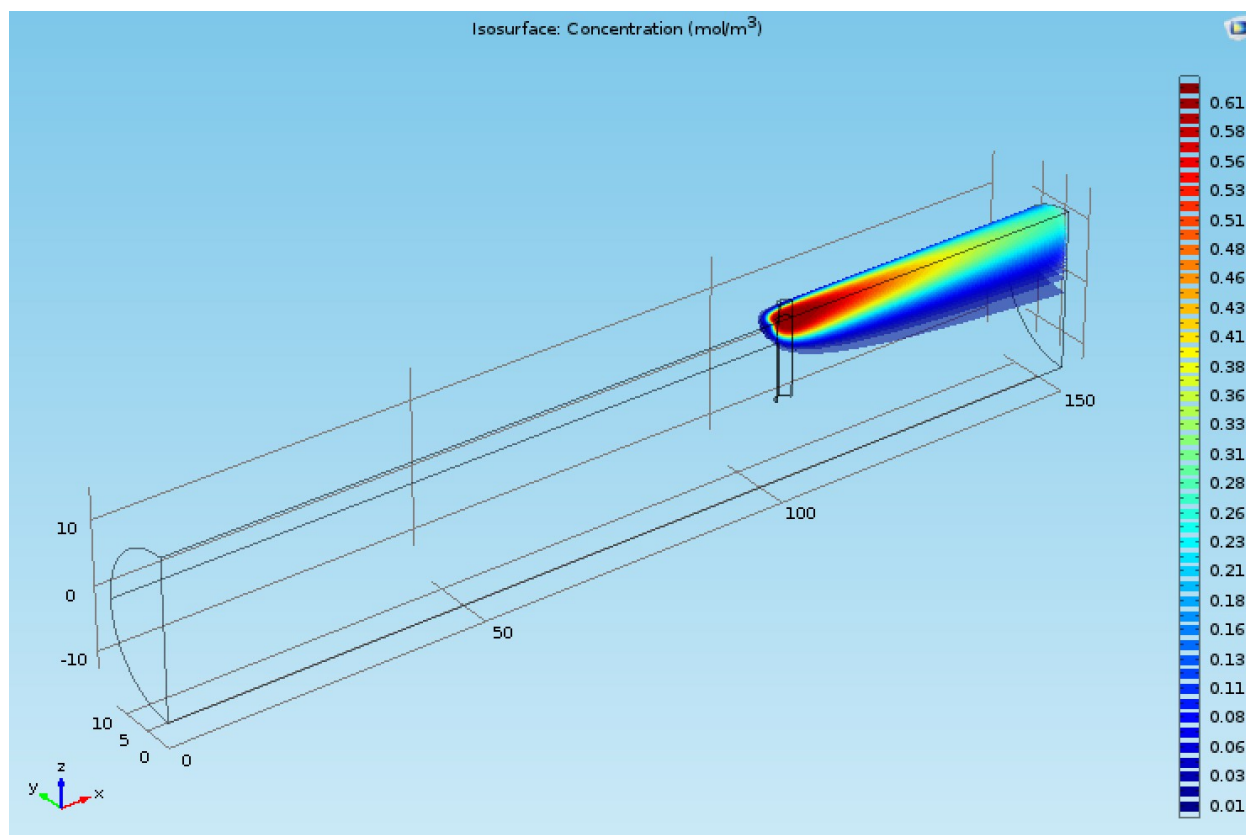


Figure 17 Distribution of dissolved oxygen concentration in the column with O₂ injection by 1.25 mm radius needle

From the figure, it could tell that the concentration of the oxygen was the highest around the injection needle, but the concentration distribution was not symmetrical in the y direction. This was because of the velocity of the water in the x direction. From the figure, it can be seen that the oxygen was not completely mixed before reaching the top of the column; as a result, the oxygen concentration was not the same at top, so this might bring errors to sampling. In this case, a proper position to make the sample was quite important.

4.4.2 Chlorobenzene diffusion in the water in the column with oxygen injection

The distribution of the fluid velocity in the column was plotted in the figure below.

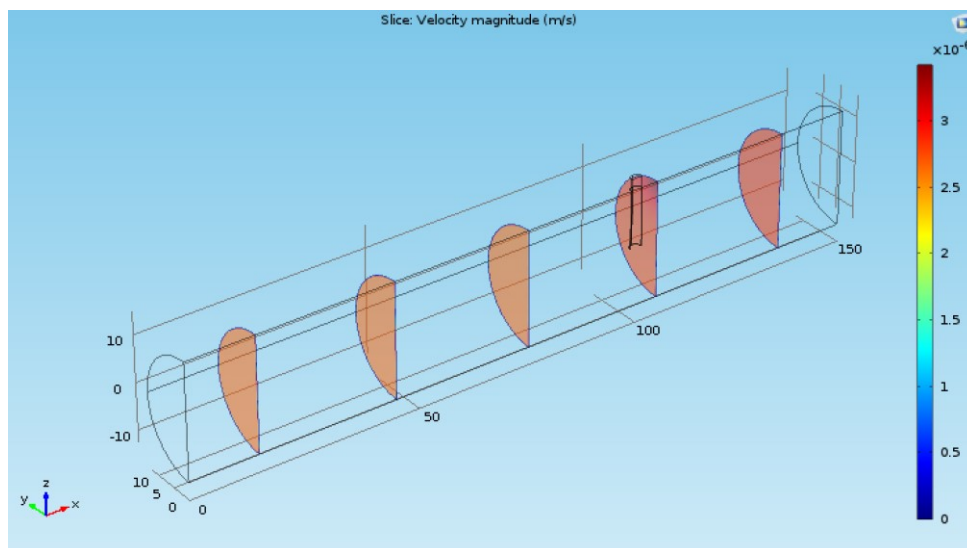


Figure 18 Distribution of chlorobenzene solution velocity in the column with O_2 injection by 1.25 mm radius needle

From the figure, the velocity of the chlorobenzene solution stayed the same as the influent velocity until it reached the injected needle area. The velocity of the fluid slightly increased as the additional oxygen was injected. In this case, the injected oxygen would not change the velocity of the solution too much.

The pressure in the column is shown below in the next figure.

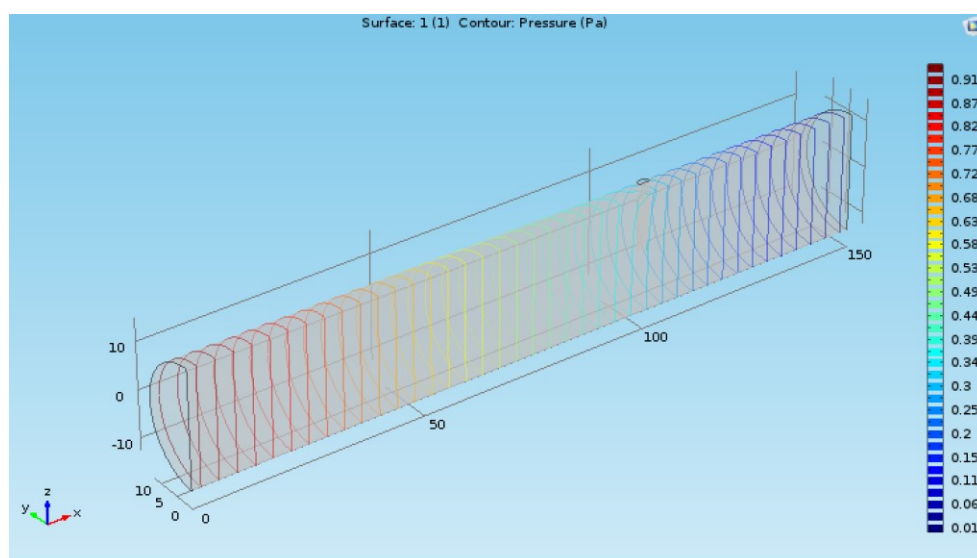


Figure 19 Pressure distribution in the column with O_2 injection by 1.25 mm radius needle

From the figure, the pressure in the column decreased along with the liquid flow in the x direction.

The distribution of the oxygen concentration is shown in the below figure.

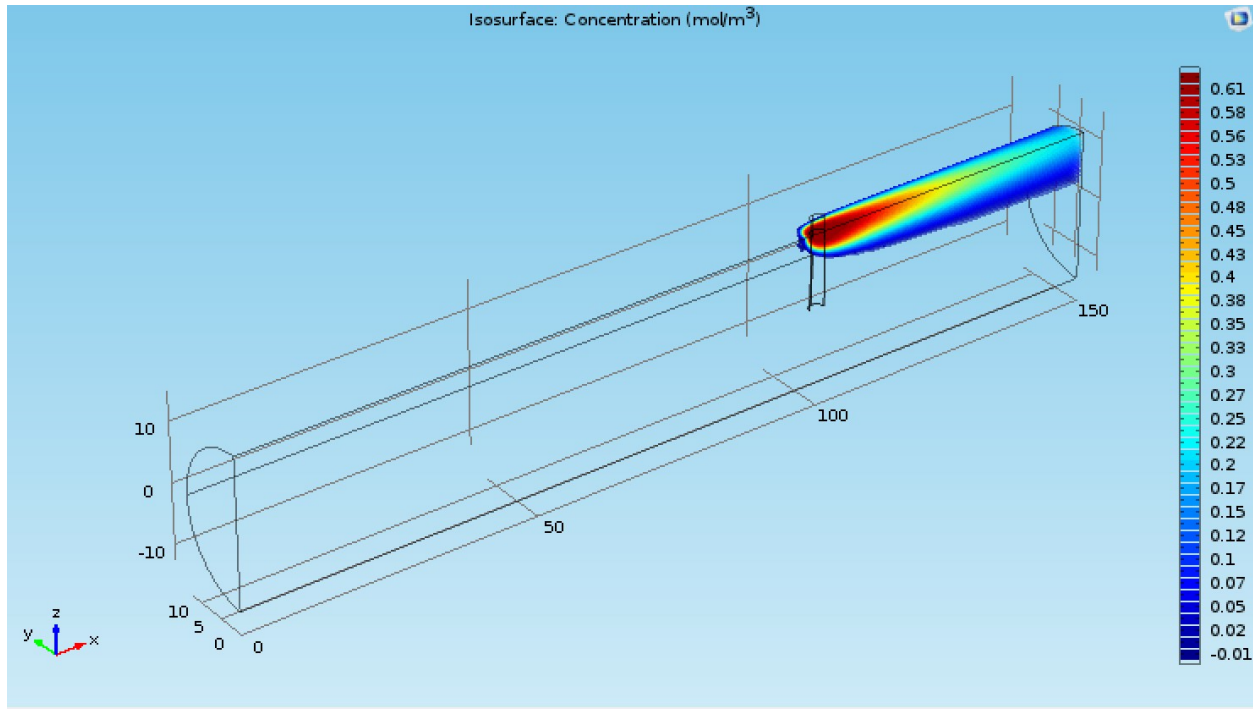


Figure 20 Distribution of dissolved oxygen concentration in the column with O₂ injection by 1.25 mm radius needle

From the figure, the concentration of the oxygen was the highest around the injection needle, but the concentration distribution was not symmetrical in the y direction. This was because of the velocity of the water in the x direction. From the figure, it can be seen that the oxygen was not completely mixed before reaching the top of the column; as a result, the oxygen concentration was not the same at top, so this might bring errors to sampling. In this case, a proper position to make the sample was quite important.

Comparing the distribution of the oxygen concentration figure in the water and with in the chlorobenzene solution. It could be seen that the concentration of the oxygen was more unified at the effluent plane in the chlorobenzene solution than in the water.

The distribution of the chlorobenzene concentration is shown in the below figure.

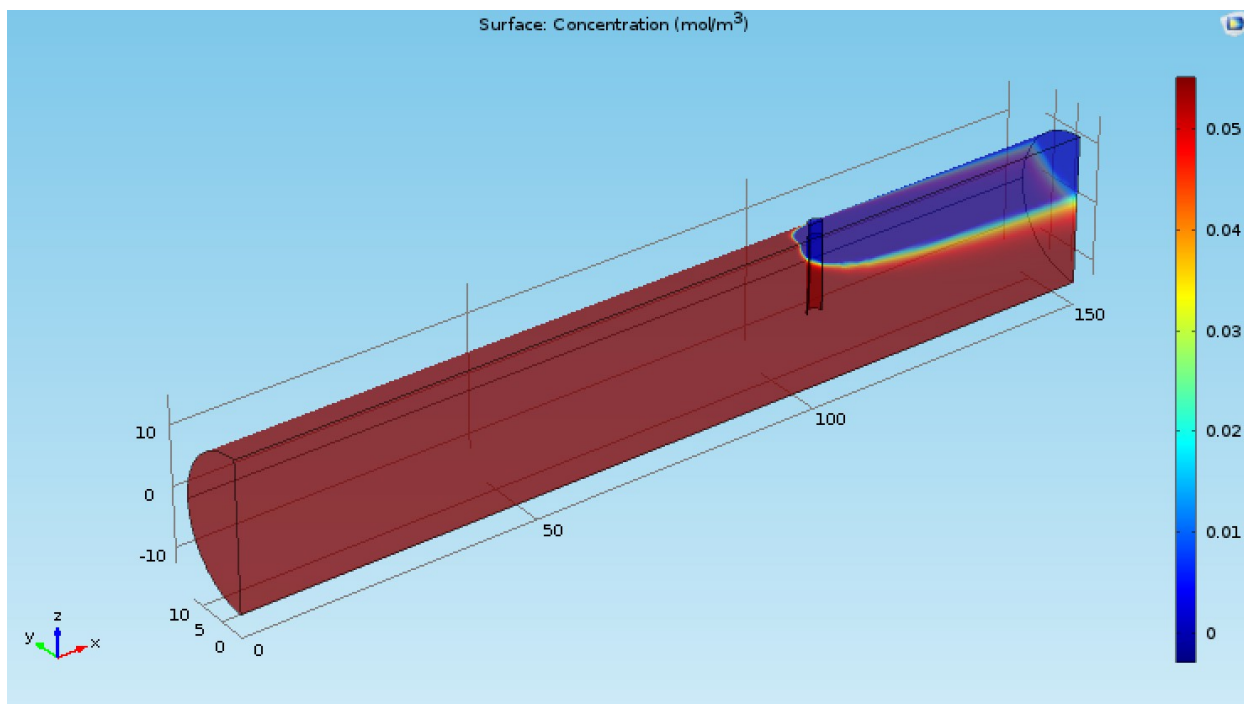


Figure 21 Distribution of chlorobenzene concentration in the column with O₂ injection by 1.25 mm radius needle

From the figure, the concentration of the chlorobenzene was the highest at the influent and remained nearly the same until around the oxygen injection point, but the concentration distribution was not symmetrical to the injection needle in the y direction. This was because of the velocity of the chlorobenzene solution in the x direction. From the figure, it can also be seen that the oxygen was not completely mixed before reaching the top of the column and the concentration of the chlorobenzene was not unified at the plane of the effluent; as a result, this might bring errors to sampling. In this case, a proper position to take the sample was quite important.

The radius of the injection needle was changed. In the figure below, the distribution of oxygen concentration is compared. The radius was 0.5 mm, 1.0 mm and 1.25 mm respectively.

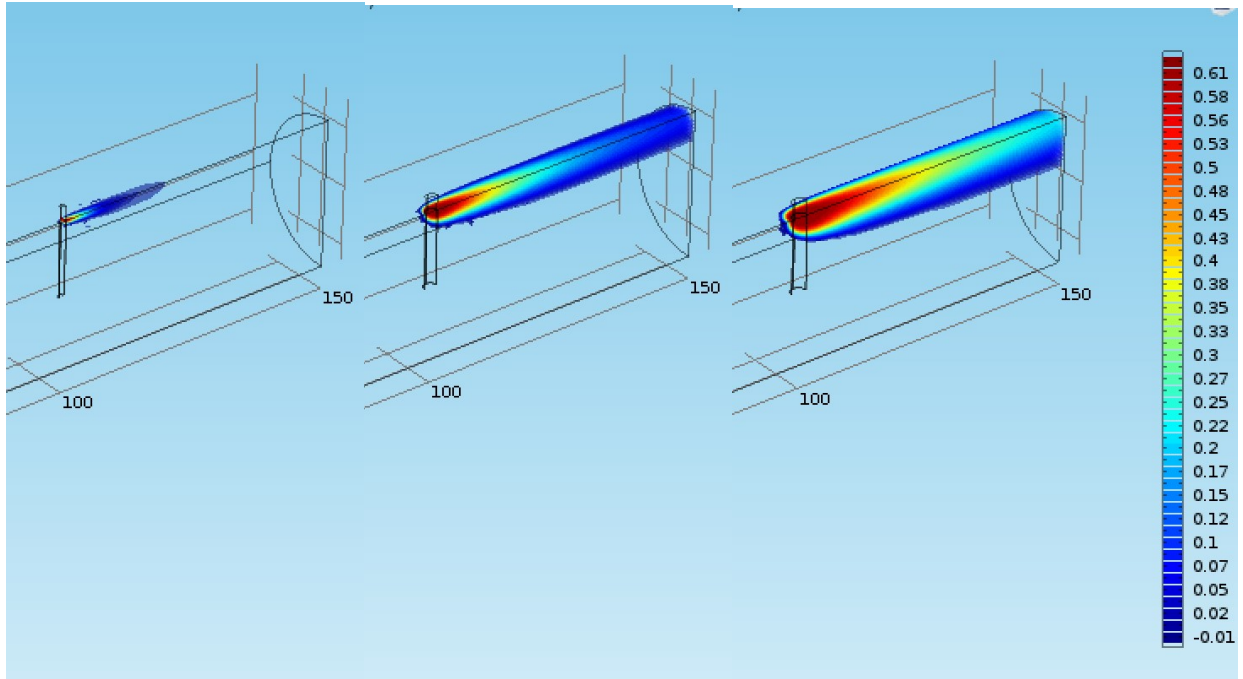


Figure 22 Distribution of dissolved oxygen concentration in the column with O_2 injection by 0.5 mm, 1.0 mm and 1.25 mm radius needle

From the figure, as the radius of the injection needle increased, the mixing of the dissolved oxygen decreased. When the radius of the injection needle was 0.5 mm and 1.0 mm, the concentration of the dissolved oxygen at the effluent plane was nearly unified. In this case, injection needles with small radius should be used in the experiment to make a well mixing of the dissolved oxygen and make it easier for sampling.

In the below figure, it contrasted the distribution of chlorobenzene concentration. The radius of the injection needle was 0.5 mm, 1.0 mm and 1.25 mm respectively.

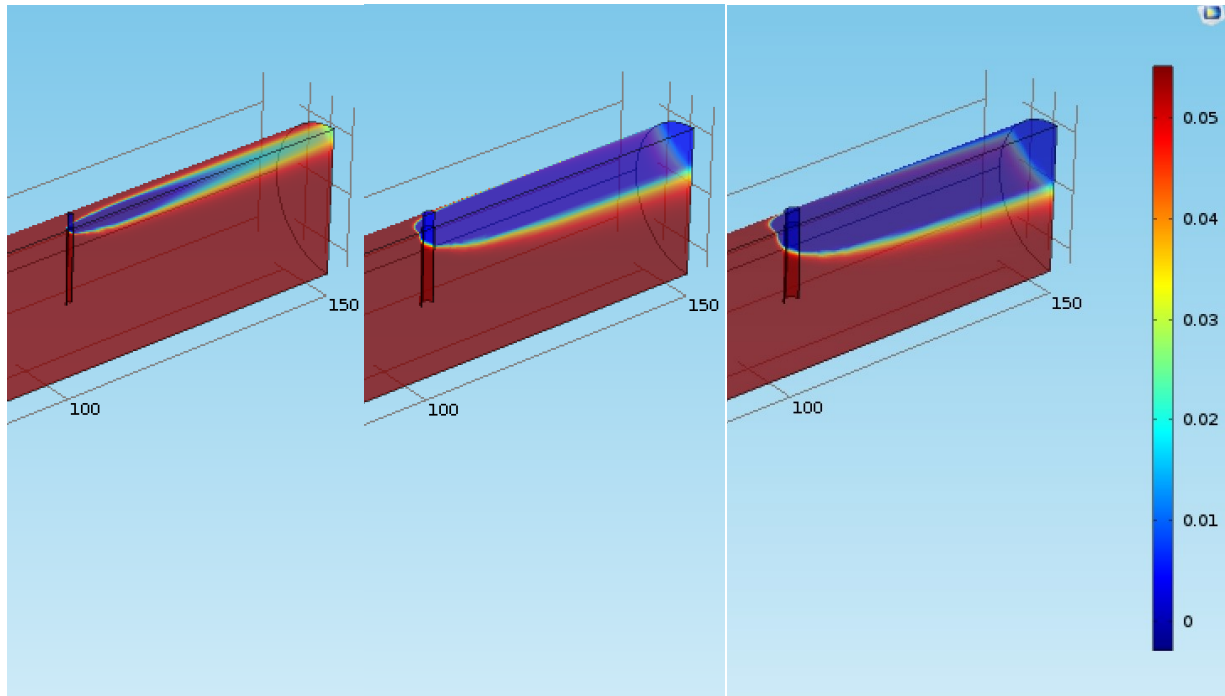


Figure 23 Distribution of chlorobenzene concentration in the column with O_2 injection by 0.5 mm, 1.0 mm and 1.25 mm radius needle

From the figure, as the radius of the injection needle increased, the mixing of the chlorobenzene decreased. However, among all three situations, none of the concentration of the dissolved oxygen at the effluent plane reach the same concentration at every point. In this case, injection needles with small radius should be used in the experiment to make a well mixing of the chlorobenzene.

CHAPTER 5 CONCLUSIONS

Based on the experiment, headspace methane injection using GC-FID is a reliable method for dissolved methane detection. The linear relationship was quite good for the concentration ranges of 0.1-1.0 mg/L, 1.0-20 mg/L and 70-4000 mg/L. In this case, the methane detection method can be applied to our experiment.

Based on the experiment, using the phenol-sulfuric acid method for quantifying the biofilm is not reliable. Although this traditional total sugar test has a quite good linear relationship in the detection range of 0.1-0.3 g/L, but the approximate concentration of our samples does not fall into this range. The linear relationship of the calibration under the concentration of 0.1 g/L was really poor. In this case, this method may not be used in quantifying the biofilm in our experiment.

When it comes to using NaS_2O_3 solution to eliminate the dissolved oxygen in the influent, the optimal setting is when the concentration of NaS_2O_3 was 0.1 M NaS_2O_3 and the flow rate was 1.618 mL/min and the liquid went to the pump first and then went to the NaS_2O_3 solution and the connections of the pipes were kept under the NaS_2O_3 solution, the O_2 removal reached the highest. In this case, too high concentration of NaS_2O_3 is not needed as the flow rate is slow enough to allow the reaction to take place. The connections should be as few as possible and should be kept in the NaS_2O_3 solution.

Based on the column modeling of the oxygen diffusion in the water and in the chlorobenzene, the place of collecting samples of the effluent does matter. In order to make the concentration distribution more unified, the radius of the injection needles should be small enough.

The challenge right now is to determine the rate constants of the chlorobenzenes biodegradation, so that the model could be applied to predict the results of the biodegradation.

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EDUCATION

Master of Science Student in Geography and Environmental Engineering (GPA: 3.6/4.0) August 2015 - Present
Department of Environmental Health and Engineering, Whiting School of Engineering, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University, Baltimore, MD Academic Advisor: Professor Lynn Roberts

Visiting Student (GPA: 3.8/4.0) June 2015 - August 2015
Stanford Environmental & Water Studies Summer (SEWSS) Program with Certificate, Stanford University, Stanford, CA

Bachelor of Science in Environmental Science (GPA: 91/100; 3.91/4.00) August 2011 - June 2015
Metallurgical Resource and Environmental Engineering Research Institute, School of Materials & Metallurgy, Northeastern University, Shenyang, Liaoning, China

Visiting Student (GPA: 3.4/4.0) June 2014 - August 2014
Summer Sessions, University of California Berkeley, CA

Visiting Student (GPA: 4.0/4.0) March 2014 - June 2014
University & Professional Study Program, University of California San Diego, CA

RESEARCH EXPERIENCE

***In situ* Bioremediation of Slow-moving Groundwater Polluted by Chlorobenzenes** May 2016 - Now
Professor Edward Bouwer, Department of Environmental Health and Engineering, Whiting School of Engineering, Johns Hopkins Bloomberg School of Public Health, Johns Hopkins University

- Conducted biological degradation of chlorobenzenes through columns which simulate flow through bio-barrier materials and natural oxygen gradients from anaerobic to aerobic conditions
- Designed multiple batch experiments to investigate the optimal conditions for biodegradation; maintained and monitored fixed-film column study simulating the *in situ* degradation process of a reactive barrier
- Developed new protocols for dissolved methane analysis using headspace injection by GC-FID and granular particle biofilm quantification using extraction and protein assays

Study on Treatment of Domestic Sewage Utilizing Algae-Sludge Symbiosis System December 2014 - June 2015
Professor Mei Wang, Environmental Chemistry Lab, Metallurgical Resource & Environment Engineering Research Institute, School of Materials and Metallurgy, Northeastern University

- Optimized the ratio of *Chlorella* sp. and active sludge in symbiosis system of domestic sewage treatment
- Utilized nutrient starvation method to further optimize cultivate conditions, improving contaminate removal rate
- Investigated the feasibility of using *Chlorella* sp. photosynthesis to replace external aeration

Separation of Cr³⁺ and Fe³⁺ in Multicomponent Acid Solution System May 2013 - March 2015
Professor Maofa Jiang, Key Laboratory for Ecological Metallurgy of Multimetallic Mineral, Ministry of Education, Northeastern University

- A key section in an innovative and Cr⁶⁺-free method of producing Cr(III) salt, in which chromite was leached by sulfuric acid instead of strong alkali with Cr⁶⁺ remaining in the slag in traditional methods
- Utilized goethite, jarosite respectively and optimized as “jarosite-goethite two-step” method to separate Fe³⁺ and Cr³⁺ and extract Fe³⁺ using P507 and P204 respectively in the sulfuric acid leaching solution of chromite

Fundamental Research of Zinc-iron Resource Utilization in Ferroalumen Residue January 2013 - March 2013
Professor Diankun Lu, Nonferrous Metallurgy Institute, School of Materials & Metallurgy, Northeastern University

- Utilized ultrasonic to accelerate Zn washing off in ferroalumen residue
- Separated Fe³⁺ and Zn²⁺ in the acid leaching solution of ferroalumen residue and produced magnetite

PUBLICATION

- J. Liu, M. Jiang, Y. Li & M. Liu. (2015). Separation of Cr^{3+} and Fe^{3+} from Sulphuric Acid Leaching Solution of Chromite by Goethite Method. *The Chinese Journal of Process Engineering*, 15(002), 242-246.

CONFERENCE PRESENTATION

- J. Liu, M. Jiang, Y. Li & M. Liu: Study on the Separation of Cr^{3+} and Fe^{3+} from Sulphuric Acid Leach Solution of Chromite by Goethite Process, in the 18th Conference on Metallurgical Reaction Engineering, Chongqing, China, October, 2014

PROFESSIONAL EXPERIENCE

Metallurgical Resource & Environment Engineering Research Institute: Shenyang, China

March 2015 - June 2015

Undergraduate Lab Teaching Assistant in Environmental Chemistry Laboratory

- Equipment and chemical purchase, management, maintenance and distribution
- Lab safety orientation and regular safety inspection; preparation and preliminary experiments for lab courses

Key Laboratory for Ecological Metallurgy of Multimetallic Mineral: Shenyang, China

May 2013 - March 2015

Lab Research Assistant in Hydrometallurgy Laboratory

- Conducted research experiments and projects; lab equipment management and maintenance

Shenyang Shenshui Gulf Sewage Treatment Plant: Shenyang, China

August 2013

Lab Analyst Intern in the Biochemical Treatment Unit

- Tested pH, TOC, COD, BOD, $\text{NH}_3\text{-N}$, SS and other indexes of biochemical treated water

Northeastern University: Shenyang, China

October 2012 - July 2013

Tutor in School of Materials and Metallurgy

- Tutored Chemistry, Probability Theory and Mathematical Statistics

Shenyang Environmental Monitoring Station: Shenyang, China

June 2013

Lab Analyst Intern in Analyzing Department

- Tested the water-quality indexes of sample water

SELECTED ACADEMIC HONORS AND AWARDS

- Second Place of 2016 Chesapeake Water Environment Association Student Design Competition 2016
- Excellent Scientific Project in the 8th National College Students Innovation and Entrepreneurship Conference 2015
- Third prize for Scientific Paper in the 20th "Challenge Cup" Liaoning Province College Students Academic Science and Technology Competition 2015
- Excellent Project in National College Students Innovation Training Program 2015
- Second prize in the engineering drawing (hand drawing and software) competition 2012
- First class scholarship of Northeastern University (3 times) 2011 - 2015
- China Aerospace Science and Technology Corporation scholarship 2013 - 2014
- Xibeitong scholarship 2012 - 2013
- Cai Guanshen scholarship 2011 - 2012

SELECTED EXTRACURRICULAR HONORS AND AWARDS

- Outstanding student of Northeastern University (3 times) 2011 - 2015
- Excellent award in English British Parliament system debate competition 2013
- Second prize in "My University Dream" English speech contest 2013
- First prize in career challenge competition 2012